FACTORS LIMITING THE EFFICACY OF DECONTAMINATION WASHES FOR FRESH PRODUCE

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ABSTRACT

Recent studies with apples and alfalfa sprouts have examined factors that limit the efficacy of hydrogen peroxide (H_2O_2) treatments in reducing bacterial populations, necessitating development of alternative procedures. Reduction of *Escherichia coli* on inoculated apples by washing with H_2O_2 would be limited by bacterial infiltration through the calyx into interior tissues of apples, or by presence of bacteria in punctures, which would preclude contact between bacteria and the wash solution. Bacterial adhesion on apple surfaces, preventing removal by washing, increased as the interval between inoculation and washing increased from 30 minutes to 48 hours. Rinsing residual H_2O_2 from fruit surfaces following a wash greatly reduced the wash efficacy. Efficacy could be improved by use of a two-stage wash in which a detergent solution was applied first, rinsed off, and then followed with a H_2O_2 wash, with no final rinse. Residual H_2O_2 gradually dissipated during product storage and subsequent processing. Attempts to decontaminate growing alfalfa sprouts by watering with 200 ppm H_2O_2 did not significantly reduce bacterial populations, and at 1000 ppm, phytotoxic effects were observed. Irradiation of seeds or sprouts with gamma radiation is a promising alternative to washing for elimination of *E. coli* O157:H7 and *Salmonella* spp. Preliminary studies of competitive exclusion of *Salmonella* indicated that pathogen growth on sprouts could be suppressed by application of an innocuous pseudomonad bacterium to the seed prior to germination.

INTRODUCTION

Previously, we reported some of our research on the efficacy of washing with dilute hydrogen peroxide solutions to reduce bacterial populations on fruits and vegetables (Sapers et al., 1997; Sapers et al., 1998). During the past year, we have encountered several problems that limit the ability of anti-microbial washes to decontaminate produce. These include the failure of conventional washing

formulations to reduce bacterial populations on produce by more than 1 or 2 $\log_{10}$ CFU, the inability of washing to reduce bacterial populations on produce if the bacteria are able to penetrate into plant tissues, and the failure of dilute hydrogen peroxide washes to have any significant effect on the bacterial load on sprouts.

Our current objectives are to systematically investigate factors that limit the effectiveness of decontamination washes for produce, and to develop alternative means of decontaminating produce where required. This report is an update on our progress in these areas.

RESEARCH ON DECONTAMINATION OF APPLES

Bacterial Adhesion to Apple Surfaces

One of the factors limiting the efficacy of washing as a means of decontaminating apples (and probably other commodities as well) is the adhesion of bacterial cells to product surfaces. We studied the rapidity and extent of *E. coli* (ATCC 25922) binding to Golden Delicious apples that were inoculated by immersion in a bacterial suspension, drained, and then held in air at 4 or 20°C for various times. In these trials, we measured the survival of *E. coli* on the inoculated apples and also the numbers of *E. coli* remaining after the apples had been washed with water at ambient temperature. The data show that during storage for 72 hours at both temperatures, there was relatively little change in the bacterial population on inoculated control apples (Table 1). When apples were held for 30 minutes after inoculation and then washed, about 90% of the bacteria could be removed, a one-log reduction. When apples were held for 24 hours after inoculation, washing reduced the population by only 1/2a log. For holding times in excess of 24 hours, washing was completely ineffective in removing bacteria. Whether this is a reflection of solely physical adhesion of bacterial cells to the apple surface or adhesion followed by biofilm formation is not clear. However, the effect is to greatly reduce the efficacy of washing.

A further complication of bacterial adhesion is the non-uniform distribution of *E. coli* (ATCC 25922) on the surface of inoculated apples. In this experiment, we removed the core from inoculated apples with a sterile cork borer prior to blending and plating. We compared the bacterial load on the surface of inoculated apples from which the cores had been removed, expressed as CFU/cm^2, with the bacterial load on the stem and calyx ends of the pooled cores (Table 2). We found that the

bacterial load per cm^2 was about 3 times greater on the external surfaces of the cores than on the remainder of the apples. The ability of *E. coli* to bind preferentially to the apple surface near the stem and calyx ends greatly complicates the problem of washing because fruit washing systems are not designed to direct jets of water or detergent solution in these areas of the apple. Such a modification of washing equipment might improve the efficacy of washing in reducing bacterial populations on apples or other commodities.

Effects of Rinsing After Application of an Anti-Microbial Wash

Rinsing to remove residues of anti-microbial agents or other treatment chemicals might be considered an essential part of any washing treatment. However, in our washing experiments with 5% hydrogen peroxide and combinations of hydrogen peroxide with commercial surfactant formulations, we found that rinsing the inoculated apples with water after use of hydrogen peroxide alone or in combination with surfactants substantially reduced the ability of the treatments to lower the bacterial population (Table 3). We believe that when washed apples were homogenized, residual hydrogen peroxide from the wet apple surfaces killed additional bacteria that adhered to inaccessible parts of the apple, for example, near the stem and calyx, during the several minutes interval before dilution and plating. Rinsing reduced the concentration of residual hydrogen peroxide from about 1000 ppm to only 20-50 ppm, not enough to exert much lethal effect.

We developed an alternative means of washing apples with surfactants and hydrogen peroxide that avoids removal of residual peroxide. Apples are washed in two stages: in the first stage, a commercial surfactant formulation is applied to remove soil and then is rinsed off. In the second stage, the peroxide solution is applied without rinsing. Residual peroxide is effective in killing surviving *E. coli* when the fruit is homogenized. Eventually, the residual peroxide breaks down to water and oxygen leaving no residue. Results of washing trials indicate that the two-stage treatment is at least as effective as a single stage wash in reducing the population of *E. coli* on inoculated apples (Table 4). These results may be applicable to commercial production of apple cider where the washed apples would be disintegrated in a hammer mill prior to pressing, a sequence analogous to our homogenization of treated apples prior to sampling for microbiological evaluation. However, the efficacy of this approach should be confirmed using *E. coli* O157:H7 in place of the non-pathogenic strains employed herein.

Effects of Contaminated Punctures

It is not unusual to find apples with skin punctures in raw material for processing or even in fruit intended for fresh market. Such damage can occur from

hail or bird pecks prior to harvest or can result from contact with stems of adjacent apples or splinters in wooden bins during harvesting and handling. If apples with skin punctures became contaminated with *E. coli*, as might occur in a flume containing contaminated water used to convey fruit within a packing plant or cider mill, bacterial growth could occur in the punctured area. We found that bacterial populations reached 10^5 CFU/g in 24-48 hours in punctured apples that were inoculated with *E. coli*, even when the initial level of inoculation was low (Table 5). The whole apple counts really represent a population in excess of 10^6 CFU/g in the area of the puncture, based on weight of excised apple flesh surrounding puncture.

As one would expect, bacteria within a puncture may not all be killed or removed by washing. Our data show how the efficacy of one or 2-stage wash treatments in reducing the *E. coli* population is limited to less than 2 $\log_{10}$ CFU (Table 6). This can be improved somewhat by addition of 1000 ppm hydrogen peroxide to the apple homogenate during sample preparation. However, it is probably not feasible to add hydrogen peroxide to disintegrated apples or juice during commercial cider production because of the complexity of the process, possible loss of quality, and regulatory constraints.

What can be done about the problem of contaminated punctures in apples? It is virtually impossible to exclude all punctured apples from a processing line because of the absence of suitable sorting equipment and the fallibility of human inspectors. Decontamination with antimicrobial washes shows little promise. A surface pasteurization treatment with hot water or steam might kill bacteria within shallow punctures without imparting an undesirable cooked flavor to the juice. Alternatively, instead of washing apples with an anti-microbial agent, the juice might be heat pasteurized by a flash pasteurization process or pasteurized by irradiation with UV light, with some possible loss of fresh flavor. Both pasteurization methods are now being used in the U.S.

Bacterial Infiltration into Apples

It is well known that when a porous commodity containing internal gases at a higher temperature is submerged in water at a lower temperature, the gases contract and produce a partial vacuum. As a result of this vacuum, water and any bacteria suspended in the water will infiltrate into the product interior where they cannot be removed by washing. This phenomenon has been reported with tomatoes (Bartz and Showalter, 1981). Data for apples immersed in a suspension of *E. coli* O157:H7 were obtained by Robert Buchanan, formerly at the Eastern Regional

Research Center and now with the U.S. Food and Drug Administration (Buchanan et al., 1998). They show that under temperature conditions favoring infiltration, the pathogen is drawn through the calyx into the apple core. In contrast, little or no infiltration occurs when cold apples are submerged in warm water. The infiltration process can be visualized by adding a dye such as Red 40 to the water and observing the penetration of the dye into the apple interior after the apple is submerged. While we have no direct evidence linking infiltration to food poisoning outbreaks, this scenario could occur when bins of apples are unloaded into water tanks or flumes to convey them into a packing or processing plant or when the apples are washed. It is imperative to avoid situations where the temperature differential between the apples and water could permit infiltration. If infiltration is likely to occur prior to apple juice production, which would make washing the fruit useless, the only recourse would be to pasteurize the juice by heat or UV irradiation.

RESEARCH ON DECONTAMINATION OF SPROUTS

Limits of Chemical Disinfection Methods

Previously, we presented data showing the failure of dilute hydrogen peroxide to substantially reduce the bacterial load on sprouts when the treatment was applied to fully grown sprouts (Sapers et al., 1997). More recently, we have examined the effects of calcium hypochlorite treatments, applied to alfalfa seeds for sprout production prior to germination, and dilute hydrogen peroxide solutions, applied during irrigation of the growing sprouts. This was done in a small-scale commercial sprout growing system. We found that treatment of alfalfa seeds with as much as 2% calcium hypochlorite had little effect on the total aerobic plate count or coliform count of the 4-day old sprouts (Table 7). Similarly, addition of 200 ppm hydrogen peroxide to the irrigation water had little or no effect on the bacterial counts, and 1000 ppm hypochlorite injured the sprouts. More recently, we showed that a buffered 3% calcium hypochlorite solution (pH 7), which contains 20,000 ppm available chlorine, did reduce the population of *E. coli* O157:H7 on inoculated seeds by about 4 logs. It is not clear whether this approach is adequate to assure a safe product or whether treatment of seeds with such a high concentration of hypochlorite would have any toxicological consequences. In all likelihood, the limited success of chemical treatments in reducing microbial loads on seeds and sprouts is due to the presence of contaminating bacteria within inaccessible cracks on seeds, which could proliferate after germination, or to incorporation of bacteria in protective biofilms on the sprout surface.

Irradiation as an Alternative Treatment for Decontaminating Seeds and Sprouts

The Eastern Regional Research Center has had a long history of research on the use of ionizing radiation to eliminate human pathogens from foods. During the past year, we have investigated use of gamma radiation to kill *E. coli* O157:H7 and *Salmonella* on alfalfa seeds and sprouts (Thayer and Rajkowki, 1998). Results indicated that irradiation could reduce the *E. coli* O157:H7 population by 4 $\log_{10}$ CFU at a dose that did not affect seed germination, an essential requirement for any seed decontamination treatment. However, *Salmonella* was more resistant to radiation, and the same dose reduced the population by only about 3 $\log_{10}$ CFU. The irradiated seeds germinated normally, although the resulting sprouts took a little longer (~ 24 h) to grow than the control sprouts. However, a dose sufficient to reduce the population of *Salmonella* by 5 logs, with a margin of safety to compensate for dose variation within a bag of seeds, might result in some damage to the seeds affecting germination. This needs to be investigated.

Populations of *E. coli* O157:H7 and *Salmonella* on alfalfa sprouts also could be greatly reduced by gamma irradiation, without injury to the sprouts. However, in the U.S., most sprout growing operations are small and widely scattered so it probably would be more practical to irradiate seeds than the sprouts.

Competitive Exclusion

Because both chemical decontamination with hypochlorite and irradiation have some limitations, we are studying a third approach to improve the microbiological quality of sprouts, the use of competitive exclusion to suppress growth of *E. coli* O157:H7 on sprouts. With this biocontrol method, a harmless species of bacteria that can occupy the same niche as the human pathogen but is antagonistic or can outgrow it, is introduced on inoculated seeds during germination. Preliminary results are very encouraging.

CONCLUSIONS

We have investigated some of the factors that limit the efficacy of washing as a means of decontaminating apples containing *E. coli*. Adhesion of this bacterium to apple surfaces is rapid, and localized areas of high population density may occur. The efficacy of a hydrogen peroxide wash in killing *E. coli* is reduced if residual peroxide is removed by rinsing. A two-stage wash treatment for apples was developed to permit use of surfactants in combination with hydrogen peroxide while at the same time retaining residual hydrogen peroxide on apple surfaces.

The presence of *E. coli* within skin punctures, where it is inaccessible to treatment and can grow, can defeat any attempt to decontaminate apples by washing. The occurrence of bacterial infiltration into the apple core will have the same effect. Under such circumstances, juice made from the apples would require pasteurization, but contaminated apples intended for fresh market could not be pasteurized and would be hazardous.

Research on the decontamination of alfalfa seeds and sprouts has established the limits of chemical treatments such as use of calcium hypochlorite and hydrogen peroxide. Washing with 3% calcium hypochlorite solution could reduce the population of *E. coli* O157:H7 on inoculated seeds by approximately 4 $\log_{10}$ CFU. Irradiation of seeds or sprouts with gamma rays could achieve similar reductions with little or no effect on germination or sprout quality. Research in progress on use of competitive exclusion to control human pathogens on sprouts is showing promise.

Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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Table 1. Adhesion of *E. coli* (ATCC 25922) on Apple Surfaces

	Log_{10} CFU/g			
	4C°		20C°	
Time after Inoculation (hr)	Inoculated Control	After Water Wash	Inoculated Control	After Water Wash
0.5	4.4 ± 0.1	3.5 ± 0.0	4.4 ± 0.0	3.4 ± 0.0
24	3.9 ± 0.1	3.2 ± 0.1	4.8 ± 0.0	4.3 ± 0.2
48	3.9 ± 0.2	4.0 ± 0.8	4.1 ± 0.2	4.6 ± 0.1
72	3.7 ± 0.2	3.6 ± 0.2	4.2 ± 0.3	3.9 ± 0.0

Table 2. Distribution of *E. coli* (ATCC 25922) on the Surface of Inoculated Apples

	Trial 1		Trial 2	
E. coli Population	Apple with Core Removed[a]	Core	Apple with Core Removed[a]	Core
CFU/g	$8.4\ 10^3$	$3.8\ 10^4$	$1.7\ 10^4$	$8.8\ 10^4$
Total CFU[b]	$1.1\ 10^7$	$6.9\ 10^6$	$2.1\ 10^7$	$1.5\ 10^7$
CFU/cm^2[c]	$6.7\ 10^4$	$1.7\ 10^5$	$1.3\ 10^5$	$3.6\ 10^5$

[a]Cored with 20 mm. diam. sterile cork borer.

[b]CFU/g x weight of cored apple or core.

[c]Total CFU/surface area of cored apple or ends of core plug.

Table 3. Effect of Rinsing on Efficacy of H_2O_2-Based Wash for Decontamination of Golden Delicious Apple Halves Inoculated with *E. coli* (ATCC 25922)

Treatment	Rinse	Log_{10} CFU/g	H_2O_2 Residue (ppm)[a]
Inoculated control	–	5.18	–
5% H_2O_2	No	2.11	690
	Yes	3.34	18
5% H_2O_2 + 1% Acidic Surfactant A	No	1.82	1040
	Yes	3.38	54
5% H_2O_2 + 1% Acidic Surfactant B	No	2.76	940
	Yes	3.15	30

[a]Determined within 2 min of sample treatment.

Table 4. Efficacy of Two-Stage Washes with Commercial Sanitizers and H_2O_2 in Decontaminating Whole Golden Delicious Apples[a]

Expt.	Treatment[b]	n	Log_{10} CFU/g Reduction[c]
A	5% H_2O_2	2	3.17 ± 0.81
	1% Acidic surfactant	2	1.95 ± 0.26
	1% Acidic surfactant; rinse; 5% H_2O_2[d]	2	3.86 ± 0.17
B	5% H_2O_2	6	2.22 ± 0.08
	4% Trisodium phosphate	6	2.08 ± 0.36
	4% Trisodium phosphate; rinse; 5% H_2O_2[d]	6	2.85 ± 1.22

[a]Inoculated with *E. coli* (ATCC 25922).
[b]1 min wash at 50°C.
[c]Based on log_{10}(CFU/g) of corresponding inoculated controls (mean=4.16 ± 0.17 for Expt. A and 4.52 ± 0.12 for Expt. B).
[d]Two-stage treatment: acidic surfactant or trisodium phosphate wash followed by 5% H_2O_2 wash with intermediate rinse.

Table 5. Growth of *E. coli* in Punctures on Inoculated Golden Delicious Apples[a]

Inoculum Strength[b]	Log_{10} CFU/g[c] Time after Inoculation (hr) 0.5	24	48
0.20	3.38	4.72	5.04
0.45	3.68	4.98	4.89
0.70	4.34	5.20	5.00

[a]1-cm deep puncture made with 3.7 mm diam. sterile nail on top of apple 2-3 cm from stem.

[b]Absorbance at 590 nm of inoculum. Corresponds to inoculum concentrations of 2.5 H 10^6, 2.1 H 10^6 and 3.4 H 10^6 CFU/mL at absorbance values of 0.20, 0.45 and 0.70 respectively.

[c]Based on weight of whole apple.

Table 6. Efficacy of H_2O_2-Based Washes for Decontamination of Punctured Golden Delicious Apples Inoculated with *E. coli* (ATCC 25922)

Treatment	Log_{10} CFU/g Reduction[a] Single Puncture[b]	Multiple Punctures[c]
Control (CFU/g)	4.88 ± 0.01	5.68 ± 0.38
5% H_2O_2	0.58 ± 0.07	0.91 ± 0.10
1% Acidic Surfactant; 5% H_2O_2	1.62 ± 0.10	ND[d]
1% Acidic Surfactant; 5% H_2O_2; 1000 ppm H_2O_2	2.59 ± 0.09	2.83 ± 0.65

[a]Based on control populations (log_{10} CFU/g) of 4.88 and 5.68 for single and multiple punctures, respectively.

[b]1-cm deep puncture made with 3.7 mm diam. sterile nail on top of apple 2-3 cm from stem.

[c]Four 1-cm deep punctures made with a 6.5 mm diameter sterile nail on opposite sides of apple on equator.

[d]ND = not determined.

Table 7. Effect of Seed and Irrigation Water Treatment on Bacterial Population of Alfalfa Sprouts

		Log_{10} CFU/g Alfalfa Sprouts		
Seed Treatment[b]	Irrigation Solution	Total Aerobes[a]	Coliforms	Yeasts & Molds
None	Tap water	8.48	8.10	4.14
0.5% NaOCl	Tap water	8.39	7.58	3.37
2% $Ca(OCl)_2$	Tap water	8.57	7.40	2.60
0.5% NaOCl	200 ppm H_2O_2	8.56	7.65	3.94
2% $Ca(OCl)_2$	200 ppm H_2O_2	8.31	7.55	3.50
0.5% NaOCl	1000 ppm H_2O_2	Sprouts injured, not marketable		

[a]Microbial loads were determined on 4-day old sprouts.
[b]All seed treatments were 10 minutes in duration.